DYNAMICA OF CHANGE IN THE OSMOTIC RESISTANCE OF ERYTHROCYTES IN HUMANS AND IN ANIMALS DURING THE PERIOD OF NATURAL ACCLIMATIZATION TO HIGH ALTITUDE

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ABSTRACT: We carried out an investigation of the osmotic resistance of erythrocytes in humans and in animals in the process of adaptation to high altitudes. We studied 15 persons and 45 rats during their time of residence at an altitude of 2000-5635 m. We established that the increase in the osmotic resistance is noted in persons during active high-altitude acclimatization and correlates with the increase in the overall tolerance of the organism to acute hypoxia and G-loads. In the absence of any special physical conditioning no regular change is observed. In animals the increase in the osmotic resistance also correlates with the increase in the overall resistance of the organism. In this case we were unable to find any regularity between the changes in the osmotic resistance and the morphological composition of the blood either in persons or in the animals.

In previously published data it was shown that in the process /171
of conditioning white rats to hypoxia in a pressure chamber or to muscular activity (swimming) an increase in the osmotic resistance of the erythrocytes began. In this case the increase in the osmotic resistance of the erythrocytes occured only in those animals in which the overall resistance of the organism was high [1-3]. This caused us to make the assumption that changes in the osmotic resistance of the erythrocytes apparently may be used as one of the tests characterizing the overall state of conditioning and resistance of an organism.

In the present article we cite data on changes in the osmotic resistance of erythrocytes in humans and in white rats subjected for a period of one month to natural high-altitude conditions (in the region of Mt. Elbrus). The work was carried out in conjunction with several other laboratories, which made it possible to compare changes produced in the osmotic resistance of the erythrocytes with changes in the overall resistance of the organism determined from the reaction to acute hypoxia and the effect of the G-loads (the investigations were conducted by Malkin and Vasil'yev), and also with changes in the morphological composition of the blood (the investigations of Blagovestovaya and Roshchina).

Method

Determining the osmotic resistance of erythrocytes in humans and in rats was carried out six times: in Moscow prior to the expedition to Terskol to an altitude of 2000 m, after 8-9, 13-14 and 26-27 days of residence in the mountains at different altitudes and finally again in Moscow during the first days after return. The blood was taken from persons (from the finger) and from the rats /172
(from the caudal vein) in the amount of 0.04 mℓ of 1% solution of NaCl. 0.02 mℓ of this sample of erythrocytes were placed into a test tube with a 5 mℓ solution of NaCl of decreasing concentration. After 30 minutes the carefully mixed samples of erythrocytes were colorimetricized in a photoelectric colorimeter of SEKN-57 type with a wavelength of 536 mμ. As a control we used the sample of erythrocytes in a 1% solution of NaCl. Such a method of change yields complete agreement between the curves of hemoysis and those which were obtained after colorimetric analysis of the centrifuged transparent solutions of hemolyzed blood samples versus distilled water. Here we felt that specially constructed experiments were necessary. Simplifying the method involved the requirements of the expedition conditions.

The values obtained for the extinctions were computed in percents of the value of the extinctions for the blood samples, completely hemolyzed. Hemolysis was assumed to be completed with comparison of the densities of the samples of erythrocytes in 2-3 strongly diluted solutions of NaCl. The values of the percent of hemolysis were plotted onto graph versus the respective concentrations of NaCl. Thus we obtained curves of the nemolysis of the blood from which we constructed curves of the distribution of erythrocytes according to their resistance, similar to that described by Gitel'zon and Terskov for acid hemolysis [14]. Along the abscissa axis we plotted the given ranges of change in saline concentration in the solution and along the ordinate we plotted the percent of increase in hemolysis of the erythrocytes for each given range of saline concentration. Thus each point along the vertical corresponds to the number of erythrocytes hemolyzed in a given interval of hypotonic solution of NaCl.

A part of the persons and animals remained constantly in Terskol at an altitude of 2000 m. The others, approximately after two weeks were shifted to Ledovaya Baza to an altitude of 3900 m and remained there for a period of two weeks. They were studied only after descent to 2000 m where the laboratory was set up.

Results

Investigations on Humans. Altogether we studied 15 persons of which 10 remained constant in Terskol at an altitude of 2000 m and were involved in ordinary occupational activity (the members of the expedition varied in sex and varied quite considerably in age),

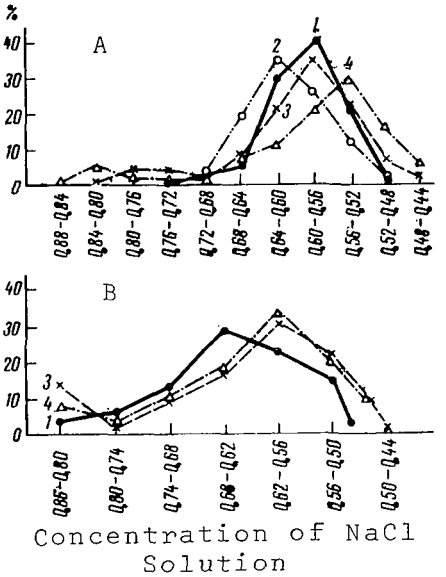
TABLE 1. HEMOLYSIS OF ERYTHROCYTES WITH DIFFERENT CONCENTRATIONS OF NaCl IN HUMANS OF THE "ALTITUDE" GROUP, %

Subject	Concentration of NaCl, %										
	0,84	0,80	0,76	0,72	0,68	0,64	0,60	0,56	0,52	0,48	0,44
Original Data in Moscow											
G.	—	—	—	0	0	5	35	70	93	100	—
M.	—	—	—	0	10	16	53	92	100	100	—
P.	—	—	—	0	0	6	40	83	97	100	—
Kh.	—	—	—	0	0	0	20	68	100	100	—
Ch.	—	—	—	0	5	9	37	77	98	100	—
$M \pm m$	—	—	—	0	3±3,3	7±3,4	37±6,8	78±5,7	98±1,7	100±0	
First Days at 2000 m											
G.	—	—	—	0	0	10	60	83	94	98	100
M.	—	—	—	0	11	40	65	86	97	100	100
P.	—	—	—	0	0	11	42	78	94	100	100
Kh.	—	—	—	0	4	22	63	85	95	99	100
Ch.	—	—	—	0	7	30	64	88	98	100	100
$M \pm m$	—	—	—	0	4±2,0	23±1,8	59±4,3	84±1,7	96±0,3	99±0,1	100±0
$P <$					0,5	0,05	0,05	Unreliable			

TABLE 1. Con'd

Subject	Concentration of NaCl, %										
	0,84	0,80	0,76	0,72	0,68	0,64	0,60	0,56	0,52	0,48	0,44
End of Residence at 3900-4200 m											
G.	—	0	3	3	4	10	34	74	94	99	100
M.	—	0	7	10	10	17	39	74	99	100	100
P.	—	0	0	3	8	12	30	58	86	96	98
Kh.	—	0	8	13	15	18	38	72	87	97	100
Ch.	—	0	4	4	8	18	41	77	94	98	100
$M \pm m$	—	0	4±1,5	7±2,1	9±1,8	15±1,7	36±2,0	71±3,4	92±2,4	98±0,2	100±0,5
$P <$			0,001	0,001	0,05	0,05	Unreliable		0,05	0,001	
In Moscow During the First Days Following Descent											
G.	0	0	6	7	8	10	11	34	75	100	100
M.	8	10	13	17	20	35	59	86	96	100	100
P.	0	0	2	5	9	19	35	59	91	98	100
Kh.	5	5	5	6	8	11	21	45	68	86	100
Ch.	4	10	12	13	14	18	22	30	70	95	100
$M \pm m$	3,4±1,5	5±2,2	8±2,1	9±2,2	11±2,4	18±3,9	29±8,2	50±1,0	80±5,7	96±2,6	100±0
$P <$			0;001	0,001	0,05	0,05	0,2	0,05	0,05	0,001	

5 persons following a 10 day residence in Terskol were gradually shifted to higher altitudes (Ledovaya Baza, 3900 m; Priyut Odinnadtsati, 4200 m) and completed ascent to the altitude of Mt. Elbrus at 5635 m. There acclimatization to high altitude from the very beginning was an active one, i.e., it involved physical labor. This was a group of males in age from 23 to 35 years. Below we shall conventionally designate this group as "altitude".



Distribution of Erythrocytes According to Osmotic Stability in Persons (A) and White Rats (B). 1. Original Values at Sea Level (Moscow); 2. First Days at an Altitude of 2000 m (Tersk 1); 3. at the End of One Month Residence at High Altitude (Terskol); 4. at an altitude of 3900 m (Ledovaya Baza). /175

Changes in the osmotic resistance of the erythrocytes were expressed most strongly in persons of the "altitude" group (Table 1.) The figure shows the distribution curves of erythrocytes based on the stability in saline solutions which vary in hypotonicity (computed from the average values in persons of the "altitude" group). In the first days of residence at an altitude of 2000 m there was noted as a rule (in 4 out of 5), a decrease in the osmotic resistance of the erythrocytes. Although the beginning and the end of hemolysis was observed with the same saline concentrations as at sea level (Moscow), i.e., at 0.72 and 0.52-0.48% respectively, but in the i - termediate saline solutions the degree of hemolysis of the erythrocytes now was higher in comparison with the original values. Thus in the range of saline concentrations from 0.60 to 0.64% on an average the amount of hemolyzed erythrocytes, as is obvious from the figure, was greater than the normal ($P<0.05$). Be the ninth day however, the values of the osmotic resistance of the erythrocytes for all saline concentrations returned to normal. At the end of residence in the mountains in persons of the "altitude" group the osmotic resistance of the erythrocytes underwent the following changes. A tendency appeared for increase in the number of the more stable

erthrocytes in the saline solution of the lowest saline concentration, (from 0.52% and below). At sea level hemolysis in two of the persons out of five ended at 0.52%, and in the other three at 0.48%, now at 0.52% the hemolysis of the erythrocytes was not ended in any of the subjects (Table 1), with 0.48% only in one, in the others only with a saline concentration of 0.44%. But simultaneously we detected a small mumber of erythrocytes on the contrary which were /176 very slightly stable, the hemolysis of which began at saline concentrations as high as 0.76%. These deviations are statistically reliable ($P<0.001$). The base of the curve of stability of the erythrocytes (see the figure) was extended; the curve had a tendency to flattening. But nevertheless the greater part of the erythrocytes in their osmotic stability did not differ strongly from the normal. Possibly this was due to the fact that determination of the osmotic resistance of the erythrocytes was carried out directly after descent from high altitude and after a very large amount of physical activity in evacuating the equipment from Ledovaya Baza. The subjects were highly fatigued and we might have expected in them certain changes in the biochemical properties of the blood which were not indifferent for the osmotic properties of the erythrocytes. It may be that these influences could counterbalance the more insignificant increase in the osmotic resistance of the erythrocytes which occured at the end of residence in the mountains and which we had determined even before the return to Moscow.

After return to Moscow and a good rest the osmotic resistance of the erythrocytes was found to be reliably different from the normal. Determinations were carried out proir to the tests of the overall tolerance to acute hypoxia and G-loads. As is obvious from the figure (A), in the blood as previously even in a still larger amount there were weakly resistant erythrocytes. Their hemolysis in three persons out of five in the "altitude" group began even with a saline concentration of 0.84%. The erythrocytes of the remaining two persons began to be hemolyzed in a solution with 0.76% (with 0.64% as the normal). However, such slightly stable erythrocytes were very few. There basic mass on the contrary possessed an increased osmotic stability. As is obvious from the figure the number of erythrocytes which hemolyze in a range of concentration from 0.60 to 0.56% is sharply decreased. The maximum of the curve is shifted significantly to the right, the number of erythrocytes which hemolyze under such low concentrations of NaCl as 0.56 - 0.44% increases significantly. Only in two persons out of the five does hemolysis cease with a saline concetnration of 0.48% and in the others this happens only at 0.44%.

The increasing osmotic resistance of the erythrocytes is in direct correlation with the increase in the overall tolerance of the organism. Prior to the expeditions into the mountains the humans of this group could barely undergo an ascent to 6000-7000 m and in this case there was observed in them gross disorders in the higher nervous activity, the EEG, the EKG and breathing (in one of

the five these disorders occurred even at 5000 m), and after a month's acclimatization in the mountains all five could remain at an al tude of 7000 m with no disruption of their functions. The tolerance to transverse G-loads was also found to be elevated (see the present collection).

Along with this the increase in the osmotic resistance of the erythrocytes in no way correlated with changes in the morphological composition of the blood. According to the data of Blagovestovaya and Roshchina, in all of the persons (although a decrease was not- /177
ed in stimulation of the hemopoiesis in the second half of residence in the mountains) in Moscow in the first days following return from the mountains the composition of the blood almost returned to the original "lowlands" value. Neither the number of erythrocytes nor the number of reticulocytes nor the composition of the hemoglobin of the blood in subjects returning from the mountains varied practically any from the normal.

Study of the osmotic resistance of the erythrocytes in the other 10 members of the expedition who differed in sex and age (from 20 years to 60) and who remained for a period of a month at an altitude of 2000 m and completed only one trip to higher altitude did not reveal any reliable deviations from the normal.

Investigations on Rats. The results obtained in investigations on 45 rats on the whole are similar to the data described for subjects of the "altitude" group. Just as in the humans of this group, and in correspondence with our previous investigations during conditioning to hypoxia in a pressure chamber, in the rats who lived for a period of 1 month on Mt. Elbrus we detected an increase in the osmotic resistance of the erythrocytes. This increase was noted also in rats who remained the entire time at an altitude of 2000 m and in rats who for the last two weeks remained at an altitude of 3900 m. Unlike changes in the osmotic resistance of the erythrocytes in persons, we found that in the rats there was no first phase of decrease on the ORE in the first days of residence in the mountains. On the contrary even of the first days there appeared a slight tendency to increase in the number of erythrocytes with increased stability as is obvious from Table 2 in which data are cited in the degree of hemolysis of erythrocytes with change in the hypotonicity of the environment and also from Figure 1 which show the curves of distribution of erythrocytes based on their osmotic stability. At the end of residence in the mountains in the first week following return to Moscow there was noted a distinct and reliable decrease in the degree of hemolysis with small concentrations of NaCl which indicates an increase in the number of erythrocytes with elevated osmotic stability. Simultaneously, just as in the humans, there appeared a slight number of osmotically very small stable erythrocytes, the hemolysis of which occurred at higher concentrations of NaCl than in the normal. The distribution curves of the erythrocytes based on their stability just as in the human were extended alon the base.

TABLE 2. HEMOLYSIS OF ERYTHROCYTES WITH VARIOUS CONCENTRATIONS OF NaCl IN WHITE RATS, %

	Concentration of NaCl Solution, %												
	0,86	0,82	0,78	0,74	0,70	0,66	0,62	0,58	0,56	0,54	0,52	0,48	0,46
Original Data in Moscow (25 Rats)													
$M \pm m$	—	0	3±0,8	9±1,9	14±1,7	30±2,4	51±2,8	72±2,4	82±1,9	88±2,1	94±1,1	100±0,1	—
First Days at 2000 m (10 Rats)													
$M \pm m$	—	—	0	1±0,2	12±0,3	27±1,6	46±2,7	67±2,6	76±2,5	85±2,2	91±1,5	99±0,3	100
$P <$				Unreliable									
At the End of Residence at 2000 m (10 Rats)													
$M \pm m$	6±2,4	11±2,8	17±3,1	22±3,7	28±4,3	36±4,2	46±3,6	62±3,1	72±3,0	81±3,1	87±2,6	95±1,6	100
$P <$			0,001	0,001	0,01	0,1	0,1	0,02	0,02	0,02	0,02	0,002	—
At the End of Residence at 3900 m (10 Rats)													
$M \pm m$	11±1,6	12±1,6	14±1,7	15±2,7	20±1,4	28±1,9	40±3,1	60±3,2	70±3,0	79±2,3	86±2,2	65±1,3	99±0,4
$P <$			0,001	0,02	0,01	0,1	0,01	0,01	0,002	0,02	0,002	0,001	
In Moscow During the First Days Following Descent (10 Rats)													
$M \pm m$	—	7±1,7	8±1,5	10±1,6	15±2,0	23±3,0	38±4,6	60±4,0	72±4,0	81±3,2	89±2,1	95±2,1	100±0
$P <$				Unreliable			0,05	0,05	0,05	0,1	0,05	0,02	0,001

It should be noted however that on the whole the increase in the ORE in the rats was expressed to a smaller degree than with experiments in conditioning to hypoxia in a pressure chamber [1, 2]. This is apparently due to the acute effect of the hypoxic stimulant. In experiments with conditioning in a pressure chamber the rats were subjected to an "altitude" of 7000 m, whereas under conditions of the described expedition the maximum altitude was 3900 m.

This increase in the ORE in the rat just as in the experiments on humans was found to be in direct correlation to the increase in the overall resistance of the organism. If the unacclimatized animals could survive at an altitude of 11,000 m for an average of 4.5 minutes, then following acclimatization to high altitude their survival was equal to 6.3 minutes, i.e., it was considerably higher (see the present collection, article by Naroshchina).

However, we could not note any distinct correlation between /179
changes in the ORE and changes in the morphological composition of the blood. Increase in the ORE at the end of residence on Mt. Elbrus still did not correlate with the increase in the hemopoesis and with counterclockwise changes in the formula of the red blood, but with return to Moscow the composition (based on the number amount of erythrocytes and reticulocytes) was practically normalized whereas the ORE remained still high.

CONCLUSION

The data obtained on changes in the ORE in the blood of healthy humans and animals acclimatized to hypoxia under natural high-altitude conditions reinforces the validity of our assumptions that determination of the ORE may be used as a test for the characteristics of the success of active conditioning in mountains accompanied by an increase in the overall resistance of the organism.

Along with this we may assume (even though the amount of material is small, it is serious in significnnce) that we can make a number of refinements in the reccomendations for using the ORE as a test for the stat of the overall conditioning of an organism. The fact is that in 10 members of the expedition we did not produce any distinct improvements in the ORE. This may be explained by the insufficiency of physical load determining the degree of which apparently is necessary in order for an altitude of 2000 m to become an effective means of raising the overall resistance of an organism/180
to changes in the ORE. But we can suggest material which shows that the correlation between the increase in overall resistance of the organism cannot always be observed. We had the possibility of determining the ORE in 4 mountain climbers who were master atheletes of high qualification, and who had been involved in mountains climbing for a period of 15 years and in recent years had made a large number of ascents. The ORE was determined in them on the 15-20th day of residence in Moscow following their return from Khan-Tengri (7000 m). Simultaneously they underwent tests of the overall re-

TABLE 3. HEMOLYSIS OF ERYTHROCYTES (in %) AT VARIOUS CONCENTRATIONS OF NaCl IN MOUNTAIN CLIMBERS 2-3 WEEKS FOLLOWING DESCENT FROM 7000 METERS

Subject	Concentration of NaCl Solution, %							
	0.76	0.72	0.68	0.64	0.60	0.56	0.52	0.48
B	0	2	4	10	19	50	100	100
S	0	0	0	5	19	62	88	100
T	0	0	2	13	25	76	100	100
Ts	0	8	8	26	54	81	94	100
M ± m	0	2±1.9	4±1.7	14±4.5	29±8.4	67±7.0	95±2.9	100±0
of the normal P <			0.5	0.25	0.5	0.25	0.5	

sistance to the effect of acute hypoxia and transverse G-loads (see the present collection). The investigation showed, as is obvious /180 from Table 3, that the ORE in the mountain climbers has no reliable deviation from the normal although the overall tolerance to acute hypoxia and G-loads was found to be significantly increased in them. Did the normalization of the ORE occur for the period of a two or three week residence at sea level, or in systematically conditioned subjects does the ORE no longer vary and is the increase in ORE in the process of conditioning characteristic only for novices? These are the questions to which we must give an answer.

It is quite possible that determining the ORE may be useful only for evaluating the positive course of the conditioning process, since the decrease in the initial period of residence in the mountains coincided with a poor state of being and the appearance of altitude illness, but the increasing ORE found in the first days following return from Mt. Elbrus to Moscow correlated with an increased tolerance of the organism.

CONCLUSIONS

1. In subjects remaining at 2000-3000 m with ascents to higher altitudes and with physical work of considerable intensity there is noted an increase in the osmotic resistance of the erythrocytes. This increase in the ORE correlates with an elevated overall tolerance of the organism which is manifested in the effect of acute hypoxia and rotation in a centrifuge and is retained after descent to sea level. No regular correlation was noted between changes in the ORE and changes in the morphological composition of the blood.

2. In persons who remain for a period of a month at an altitude of 2000 m (Terskol) and who did not undergo any special physical conditioning no regular change was noted in the osmotic resistance of the erythrocytes.

3. When white rats remained for a month at altitudes of 2000- and 3900 m this led to an increase in the osmotic resistance of the erythrocytes correlating with the increase in the overall resistance of the organism which appeared during tests in the pressure chamber with a high evacuation of atmospheric air and during rotation in a centrifuge. We were not able to find any regular correaations between the increase in ORE and changes in the morphological composition of the blood.

4. In mountain climbers of high qualification 2-3 weeks following their return from Khan-Tengri (7000 m) the osmotic resistance of the erythrocytes in no way differed from the normal which is characteristic of residents at sea level.

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